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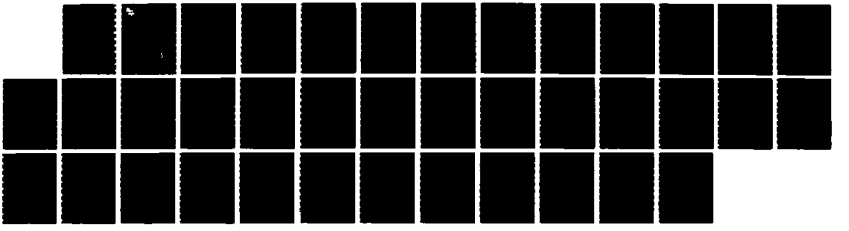

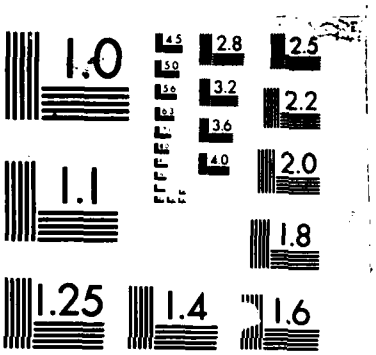

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DIFFERENTIAL EFFECTS OF GLUCAGON, INSULIN AND EPINEPHRINE ON IN VIVO GLUCOSE OXIDATION AND HEPATIC ENZYME ACTIVITY IN THE RAT

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Differential effects of glucagon, insulin and epinephrine on in vivo glucose oxidation and hepatic enzyme activity in the rat--Klain and Herman

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

Edwin S. Seaton

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In vivo oxidation of specifically labeled ^{14}C-glucose and the activity of selected hepatic enzymes involved in glucose utilization were studied in rats after the administration of glucagon, insulin or epinephrine. Compared to the controls, glucagon doubled the oxidation of U-^{14}C-glucose and enhanced 6-^{14}C-glucose or 2-^{14}C-glucose oxidation by about 83%, but did not affect oxidation of 1-^{14}C-glucose. Administration of insulin enhanced oxidation of U-^{14}C-glucose by 68%, 1-^{14}C-glucose by 71%, 2-^{14}C-glucose by 73%, with no effect on 6-^{14}C-glucose oxidation. Eprinephrine enhanced oxidation of

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6-^{14}C-glucose by 38% and had no effect on 1 Or 2-^{14}C-glucose oxidation.

Insulin and glucagon produced rapid reciprocal changes in the activities of certain glycolytic and gluconeogenic enzymes and in the activity of acetyl CoA carboxylase, a key lipogenic enzyme. Insulin produced a rapid increase in hepatic glucokinase, phosphofructokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase, and a rapid decrease in glucose-6-phosphatase and and fructose-1, 6-diphosphatase. Glucagon produced a rapid but reciprocal response in the activity of these enzymes. In addition, glucagon enhanced the activity of phosphoenolpyruvate carboxykinase. Epinephrine effects were similar to those produced by glucagon. Both glucagon and epinephrine increased the level of cyclic AMP. The results of this study suggest that glucagon and epinephrine stimulate the activity of the tricarboxylic acid cycle, whereas insulin enhances the pentose cycle. It appears that in vivo glucose utilization depends on the relative concentrations of glucagon, insulin and epinephrine in the target issues.

ABSTRACT

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In vivo oxidation of specifically labeled ^{14}C-glucose and the activity of selected hepatic enzymes involved in glucose utilization were studied in rats after the administration of glucagon, insulin or epinephrine. Compared to the controls, glucagon doubled the oxidation of U-^{14}C-glucose and enhanced the oxidation of 6-^{14}C-glucose and 2-^{14}C-glucose oxidation by about 83%, but did not affect oxidation of 1-^{14}C-glucose. Administration of insulin enhanced oxidation of U-^{14}C-glucose by 68%, 1-^{14}C-glucose by 71%, and 2-^{14}C-glucose by 73%, with no effect on 6-^{14}C-glucose oxidation. Epinephrine enhanced oxidation of 6-^{14}C-glucose by 38% and had no effect on 1- or 2-^{14}C-glucose oxidation.

Insulin and glucagon produced rapid reciprocal changes in the activities of certain glycolytic and gluconeogenic enzymes and in the activity of acetyl CoA carboxylase, a key lipogenic enzyme. Insulin produced a rapid increase in the activity of hepatic glucokinase, phosphofructokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase, and a rapid decrease in the activity of glucose-6-phosphatase and fructose-1,6-diphosphatase. Glucagon produced a rapid but reciprocal response in the activity of these enzymes. In addition, glucagon enhanced the activity of phosphoenolpyruvate carboxykinase. The effects of epinephrine were similar to those produced by glucagon. Both glucagon and epinephrine increased the level of cyclic AMP. The results of this study suggest that glucagon and epinephrine stimulate the activity of the tricarboxylic acid cycle, whereas insulin enhances the pentose cycle. It appears that in vivo glucose utilization depends on the relative concentrations of glucagon, insulin and epinephrine in the target tissues.

PREFACE

A comprehensive study to delineate metabolic alterations underlying pathologic and nutritional hypoglycemia in humans was initiated in 1979 in the Departments of Nutrition and of Medicine, LAIR. However, due to changes in the research program and the mission of the Institute, only the preliminary phase of the study was completed. The initial phase was concerned with hormonal effects on glucose metabolism in the laboratory rat. The results are presented in the following report.

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INTRODUCTION

Glucose is an optional substrate for tissues such as muscle, adipose or liver as these tissues can also use fatty acids to satisfy their energy needs. Glucose, however, is an obligate substrate for the central nervous system, since under physiological conditions, alternative substrates either are excluded by the blood-brain barrier or circulate at concentrations too low to be taken up in substantial quantities. Because the brain can neither synthesize nor store adequate supply of glucose, normal cerebral function requires a continuous supply of glucose from the circulation. Consequently, maintenance of the plasma glucose concentration above some critical level is essential to the survival of the brain and thus, the organism.

Plasma glucose levels in the mammalian organism are maintained by a series of coordinated enzymatic reactions involving hepatic synthesis and breakdown of glycogen. Many of the reactions are reversible and common to the synthetic and degradative pathways. These reactions are always close to equilibrium so that the rate and direction of flow of glucose carbons can be changed by variations in the concentrations of substrates and/or products of the reactions. However, some reactions of the glycolytic pathway are displaced far from equilibrium. They release a considerable amount of energy as heat, and therefore, cannot be easily reversed. To ensure the flow of substrates in the reverse reaction, energy barriers are bypassed by other reactions which are also irreversible and are catalyzed by different enzymes. The antagonistic reactions in the glycolytic and the gluconeogenic pathways operate simultaneously but at different rates. The irreversible reactions between glucose and pyruvate are: (a) the interconversion of glucose and glucose-6-phosphate, catalyzed by glucokinase and glucose-6-phosphatase; (b) the interconversion between fructose-6-phosphate and fructose-diphosphate where phosphofructokinase is opposed by fructose-diphosphatase and (c) two separate reactions at the pyruvate kinase bypass. Pyruvate is carboxylated to oxaloacetate in a reaction involving ATP hydrolysis and catalyzed by pyruvate carboxylase. Conversion of oxaloacetate to phosphoenolpyruvate involves a decarboxylation and phosphate transfer from either guanosine triphosphate or inosine 5'-triphosphate, which is catalyzed by phosphoenolpyruvate carboxykinase.

The activity of these key enzymes may be modulated by various metabolic mechanisms, including the action of hormones. Hormonal effects may be short-term or long-term. The former may be achieved by affecting enzyme activity without changing the amount of enzyme present. The latter may meet the functional needs of the organism by producing additional enzyme by biosynthesis and by changing the degradation rates. These metabolic control mechanisms enable the organism to remove and conserve glucose efficiently from the blood stream when the intake exceeds the demand and to rapidly form glucose from non-carbohydrate sources when the need arises. The main hormones affecting the key enzymes of glucose metabolism are insulin, glucagon and epinephrine, and on a slower time scale, the growth hormone and adrenal corticoids (1).

In the course of glucose metabolism via the major metabolic pathways, specific glucose carbons can be oxidized and expired as CO_2. These observations have been utilized to estimate the contribution of the pentose cycle to glucose metabolism (2). Several studies have demonstrated that the hormones have marked effects on glucose oxidation and that these effects are associated with specific tisues or organs. Thus, glucagon reduces U-C^{14}-glucose oxidation both in perfused rat liver (3,4) and in rat liver slices (5,6) but stimulates glucose oxidation in perfused rat heart (7). The hormone has no effect on glucose oxidation in adipose tissue (8). In contrast to glucagon, insulin consistently stimulates glucose oxidation in several systems. The hormone enhances U-C^{14}-glucose oxidation in rat epididymal adipose tissue (9), but oxidation of C-1 of glucose is stimulated to a greater extent than oxidation of C-6 (10,11). Similar observations were reported in studies with mammary gland preparations (12). Administration of insulin to diabetic rats restores hepatic oxidation of 1-C^{14}-glucose and 6-C^{14}-glucose to the level observed in control rats (13). The hormone also stimulates oxidation of U-C^{14}-glucose in rat diaphragm preparations (14,15). Exogenous insulin temporarily enhances in vivo oxidation of U-C^{14}-glucose in fed, fasted, or refed rats (16,17) and of 1-C^{14}-glucose in fed rats (18). Epinephrine decreases U-C^{14}-glucose oxidation in liver slices from fed, fasted, or refed rats (5). No epinephrine effect was observed in rat thymocytes (19).

Beyond these observations, little is known about the impact of the three hormones on glucose oxidation in the intact animal. Since these hormones regulate the major metabolic pathways of glucose, we hypothesized that the hormones would have pronounced effects on in vivo oxidation of specific carbons of glucose molecules. Accordingly, in the present study, we examined acute effects of insulin, glucagon and epinephrine on oxidation of U-C^{14}-glucose, 1-^{14}C, 2-^{14}C and 6-^{14}C. In addition, acute hormonal effects on selected strategic hepatic glycolytic and gluconeogenic enzymes were determined.

MATERIAL AND METHODS

Animals and Anesthesia - Male Holtzman rats weighing 270-300 gm were used in all experiments. They were individually housed at 25°C in stainless steel wire cages and fed a commercial laboratory rat diet. Food and water were available at all times, unless otherwise indicated. The rats were anesthetized with pentobarbital (50 mg/kg) administered intraperitoneally and used 10 minutes later for further experimentation. The degree of anesthesia appeared uniform, as none of the rats woke up and became active during experimentation.

Hormone and ^{14}C-glucose Administration - All hormone solutions and ^{14}C-glucose dissolved in saline were administered via the tail vein. Crystalline glucagon (Eli Lilly and Co., Indianapolis, Ind) was dissolved (1 mg/ml) in the manufacturer's diluent which contained 14 mg/ml lactose, 1.6% glycerol, and 0.2% phenol. Insulin solution (Iletin^R, 40 U/ml) was further diluted, and epinephrine bitartrate was dissolved in sterile saline. Where applicable, glucagon was administered first, followed immediately by insulin. Preliminary experiments indicated that single injections of 3 ug of epinephrine caused acute pulmonary edema, and the rats expired within 3 to 5 minutes. This problem was not encountered when 2 ug of epinephrine were administered over a 1-minute period. Control rats were injected with the corresponding volume of the diluent. The group sizes, dosage levels of the hormones, and the position of the label in the glucose molecule are indicated in the tables. All blood samples were obtained from the tail vein.

Hormonal Effects on Plasma Glucose Levels - A series of experiments were conducted as follows: After 0.1-0.2 ml of blood were collected for glucose analysis (zero time), 0.2 ml glucagon, insulin or epinephrine were injected and flushed into the vein with 0.2 ml of saline. Additional blood samples were taken 5, 10, 20, 30 and 40 minutes later. The blood was centrifuged at 1600 x g in a refrigerated centrifuge to obtain plasma samples. Plasma glucose levels were determined by the glucose oxidase method (20).

Hormonal Effects on Glucose Oxidation - Specifically labeled C^{14}-glucose solution was administered immediately following glucagon, insulin or epinephrine injections. The rat's head was placed in a small plastic bottle from which the bottom had been removed. Air was drawn through the bottle and expired $C^{14}O_2$ was collected in an aqueous solution of 2% sodium hydroxide. Aliquots of the solution were withdrawn and diluted with 10 ml of aqueous scintillation solution. Radioactivity was determined in a Packard scintillation spectrometer. At the end of the collection period, blood was withdrawn into heparinized syringes and processed as before.

Hormonal Effects on Enzyme Activity - After the abdomen was opened, approximately 50 mg of hepatic tissue was immediately removed (zero time) and cooled in ice-cold saline. A small piece of gauze was placed over the incision and kept moist with saline to prevent drying of the surface of the liver. Additional liver samples were collected 5, 10, 20 and 40 minutes after the hormonal injection. All liver samples were randomly taken from different peripheral sites of the organ. No samples contiguous to a previous sampling site were taken. Ten percent liver homogenates were prepared in 0.25 M sucrose solution, using an all-glass tissue homogenizer. An aliquot of the homogenate was centrifuged for 30 minutes at 105,000 x g in a refrigerated centrifuge. Enzyme assays were performed on the diluted homogenate or on a clear supernatant fluid. The following methods were applied for the determination of enzyme activities: glucose-6-phosphatase, the method of Cori and Cori (21); glucokinase, the method of Vinuela et al (22); fructose-1,6-diphosphatase, the method of Racker (23); phosphofructokinase, the method of Lea and Walker (24); pyruvate kinase, the method of Bucher and Pfleiderer (25); phosphoenolpyruvate carboxykinase, the method of Nordlie and Lardy (26) and Shrago and Lardy (27); glucose-6-phosphate denydrogenase, the method of Horecker et al (28); acetyl CoA carboxylase, the method of Hsu et al (29). Cyclic AMP was measured by the method of Gilman (30) and protein was determined by the method of Lowry et al (31). Enzyme activity was expressed as nanomoles of substrate metabolized per minute per mg of protein(nmol/min.mg). All data were initially evaluated with analysis of variance and significant differences between means were identified by the Newman-Keuls test (32).

RESULTS

The Hormones and Plasma Glucose Levels - From the zero time to 5 minutes after administration of glucagon, the glucose level increased by approximately 48% (Table 1). The maximal effect occurred 10 minutes after glucagon administration. A marked hyperglycemia was maintained for an additional 30 minutes. The glucose level 5 minutes after insulin administration decreased by about 52%, when compared to that at the zero time. Thereafter, severe hypoglycemia was observed throughout the entire 40-minute experimental period. Plasma glucose levels increased by approximately 25% in 5 minutes after epinephrine administration. Again, the levels remained elevated during the entire experimental period.

The Hormones and Glucose Oxidation - In general, the effects of glucagon and insulin on the oxidation of various glucose carbons were rapid. The effects were apparent 5 minutes after an administration of either hormone, and persisted throughout the entire experimental period (Table 2). In comparison to the controls, glucagon doubled the $^{14}CO_2$ production from U-^{14}C-glucose, and insulin increased oxidation by about 68% during the 40-minute experimental period. When glucagon and insulin were administered in combination, no additive effect on glucose oxidation was observed (Table 2). The effect of each hormone on plasma glucose was as expected: glucagon increased the glucose level and insulin decreased it. Insulin, when given together with glucagon, reduced the glucose level.

Glucagon had no significant effect on 1-^{14}C-glucose oxidation. In contrast, insulin enhanced oxidation by about 71% over the control values. No effect by insulin was observed when glucagon and insulin were administered in combination, indicating that glucagon reversed insulin's effect on 1-^{14}C-glucose oxidation. Each hormone enhanced oxidation of 2-^{14}C-glucose, glucagon by about 80% and insulin by about 68%, when compared to the control values. No further effect was observed when the hormones were administered in combination. Glucagon increased oxidation of 6-^{14}C-glucose by 83% over the controls; insulin had no effect. Glucose oxidation remained elevated even after administration of both hormones. Thus, insulin did not overcome glucagon effect on 6-^{14}C-glucose oxidation. In general, the effects of the two hormones on the oxidation of various glucose carbons were rapid. The effects were apparent 5 minutes after an administration of either hormone, and persisted throughout the entire experimental period.

Table 3 summarizes the effect of epinephrine on the oxidation of specific carbons of glucose. Compared to the control values, epinephrine did not affect 1-^{14}C- or 2-^{14}C-glucose oxidation. The hormone did enhance oxidation of 6-^{14}C-glucose by about 27% over the controls. For each instance, however, the hormone increased plasma glucose levels by approximately 22% over the control values.

The data in Table 4 show that fasting alone decreased the oxidation of 1-^{14}C-glucose by about 57% when compared to data for the fed animals (Group 3 vs Group 1). Glucagon had no effect on the oxidation of 1-^{14}C-glucose either in the control or in the fasting rats. Fasting animals depressed the oxidation of 6-^{14}C-glucose by approximately 97% when compared to fed animals (Group 7 vs. Group 5). As expected, glucagon enhanced 6-^{14}C-glucose oxidation in fed rats (Group 6 vs. Group 5). The hormone also stimulated 6-^{14}C-glucose oxidation in fasted rats (Group 8 vs. Group 7). However, this effect was less pronounced than the effect observed in fed rats. Glucagon increased plasma glucose levels in fed rats. In contrast, the hormones had no effect on glucose levels in fasting rats.

The Hormones and Enzyme Activity - Glucagon. Within 5 minutes after injection of the hormone, there was a significant increase in the activities of the key gluconeogenic enzymes, glucose-6-phosphatase, fructose-1,6-diphosphatase and phosphoenolpyruvate carboxykinase (Table 5). In contrast, the hormone reduced the activities of two key glycolytic enzymes, pyruvate kinase and phosphofructokinase and the activities of glucokinase and acetyl CoA carboxylase. The effect of glucagon persisted for at least forty minutes. The activity of glucose-6-phosphate dehydrogenase did not change. Five minutes after glucagon administration, a fourfold increase in the concentration of cyclic AMP was observed. This effect persisted throughout the entire 40-minute experimental period.

The Hormones and Enzyme Activity - Insulin The hormone produced changes in enzyme activity reciprocal to those produced by glucagon (Table 6). Glucokinase, phosphofructokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase and acetyl CoA carboxylase activity increased significantly within five minutes of insulin administration. In contrast, glucose-6-phosphatase and fructose-1,6-diphosphatase activity decreased. The insulin effect persisted for at least 20 minutes. The hormone had no effect on the levels of cyclic AMP or the activity of phosphoenolpyruvate carboxykinase.

The Hormones and Enzyme Activity - Epinephrine - Epinephrine administration produced a rapid, significant increase in the activity of fructose-1,6-diphosphatase and phosphoenolpyruvate carboxykinase (Table 7). The hormone reduced the activity of glucokinase, phosphofructokinase, pyruvate kinase and acetyl CoA carboxylase. The effect of epinephrine persisted for at least 10 minutes. The hormone had no effect on the activity of glucose-6-phosphatase or glucose-6-phosphate dehydrogenase. Five and ten minutes after epinephrine administration, a twofold increase in the level of cyclic AMP was observed.

DISCUSSION

There are many difficulties inherent in interpreting pathways of glucose metabolism on the basis of $^{14}CO_2$ production from specifically labeled glucose in a metabolically active single tissue (2). These difficulties are magnified enormously when one attempts to interpret a similar set of data from in vivo studies. It is well established that administration of any of the three hormones evokes compensatory changes in systemic levels of other hormones and metabolites, which in turn, markedly alter a number of metabolic parameters (33). Thus, during the 40-minute experimental period, a variety of compensatory changes may have taken place which could have complicated the interpretation of the observed changes. Furthermore, the contribution of individual organs or tissues to the overall production of $^{14}CO_2$ is unknown. Presumably, the most significant hormonal effects occurred in the liver, muscle, and kidney.

In the course of ^{14}C-glucose catabolism through the glycolytic pathway, pyruvate can enter the tricarboxylic acid (TCA) cycle via two different pathways: (a) by condensation with CO_2, catalyzed by pyruvate carboxylase, to form oxaloacetate, or (b) by oxidative decarboxylation, catalyzed by pyruvate dehydrogenase, to form acetyl CoA. In the latter step C-3 and C-4 of glucose are released as $^{14}CO_2$. In the TCA cycle, isocitric dehydrogenase and alpha-ketoglutaric dehydrogenase release C-2 and C-5 of glucose during the second turn of the cycle and C-1 and C-6 during the third and subsequent turns. In the conversion of oxaloacetate to phosphoenolpyruvate, catalyzed by phosphoenolpyruvate carboxykinase, C-2 and C-5 are released as $^{14}CO_2$ after the first turn of the cycle, but as C-1 and C-6 in the subsequent turns. In the pentose phosphate pathway, catalyzed by phosphogluconate dehydrogenase, C-1 of glucose is released as $^{14}CO_2$. In the subsequent reactions, the remaining $^{14}CO_2$ is metabolized as pyruvate through the TCA cycle (Figure 1). The rate at any moment at which $^{14}CO_2$ is produced is determined by the specific radioactivity of the precursor carbon destined to form $^{14}CO_2$ and the flux through the decarboxylation reactions.

Increased in vitro formation of $^{14}CO_2$ from C-6 relative to C-1 glucose has been interpreted as increased activity of the TCA cycle (2). Thus, the results of our study suggest that glucagon enhances the TCA cycle turnover. As the hormone reduces the activity of acetyl CoA carboxylase, the key enzyme in the synthetic pathway of fatty acids, an increased flux of pyruvate through the TCA cycle enhances C-6 oxidation. In the fasting animal, the TCA-cycle turnover is markedly decreased and pyruvate enters the cycle primarily as oxaloacetate (34), thus increasing the amount of cycle intermediates. With respect to the economy of the starving organism, increasing the amount of the TCA-cycle intermediates from pyruvate and therefore, ultimately from carbohydrate, makes sense, since these intermediates can be used for synthesis of amino acid and proteins. This process further dilutes the radioactivity in $^{14}CO_2$. The fed rat has no need to manufacture amino acids; it can therefore afford to burn the pyruvate directly through decarboxylation and reactions of the TCA cycle. Therefore, a large fraction of $^{14}CO_2$ observed in fasting is likely produced by conversion of oxaloacetate to phosphoenolpyruvate, catalyzed by phosphoenolpyruvate carboxykinase which responds to glucagon (Table 5). Another avenue of glucose catabolism is the glucuronic acid pathway, a pathway in which C-6, but not C-1, is oxidized to CO_2 (35). However, in this study no effect of glucagon on $^{14}CO_2$ formation from U-^{14}C-glucuronate was observed. It would appear that no significance can be attached to the contribution of the glucuronic acid pathway to the formation of $^{14}CO_2$ from 6-^{14}C-glucose.

Increased oxidation of 1-^{14}C-glucose induced by insulin confirms similar findings of others (16,18) and suggests an increased activity of the pentose cycle. This conclusion is consistent with the effect of insulin on the activity of glucose-6-phosphate dehydrogenase.

Both insulin and glucagon stimulate oxidation of 2-^{14}C-glucose. It has been demonstrated that rearrangement of glucose carbons occurs via the pentose cycle (36). C-2 of glucose is randomized into C-1 and C-3 of fructose-6-phosphate and therefore into C-1 and C-3 of glucose-6-phosphate upon the completion of the cycle. C-1 of glucose-6-phosphate is oxidized to CO_2 in the pentose cycle and C-3 in the TCA cycle (Figure 1). Indeed, in vitro studies demonstrated that the greatest randomization of glucose carbons in the pentose cycle occurs in the presence of insulin (37).

Both epinephrine and glucagon stimulate oxidation of 6-^{14}C-glucose. However, the stimulation by epinephrine was less pronounced than that by glucagon. In this respect, the action of the two hormones is similar to their effect on the activity of several gluconeogenic enzymes (Table 7, ref 38). Factors such as the dosage level of epinephrine, the half-life of the hormone, or possibly different physiologic mechanisms may account, at least in part, for the less pronounced effect of epinephrine, in comparison to glucagon, on 6-^{14}C-glucose oxidation.

Alterations in the glucose oxidative pathways were accompanied by pronounced changes in the activities of glycolytic and gluconeogenic enzymes. In general, glucagon and epinephrine stimulated gluconeogenesis and decreased glycolysis, whereas insulin enhanced glycolysis and synthesis of fatty acids and reduced gluconeogenesis. The rapid and antagonistic effects of the three hormones on carbohydrate metabolism are consistent with the previous studies of insulin, glucagon and epinephrine on these pathways (5,6,38,39).

Since the rate-limiting step in the glycolytic pathway in the liver is a phosphofructokinase step, the rapid change in the activity of this enzyme would explain the effects of glucagon and insulin on glycolysis. The activities of other glycolytic enzymes, glucokinase and pyruvate kinase are also rapidly changed in a reciprocal manner by insulin and glucagon (Figure 2,3). Glucagon and epinephrine act as inducers of the key gluconeogenic enzymes glucose-6-phosphatase, fructose-1,6-diphosphatase and phosphoenolpyruvate carboxykinase (Figure 3,4). In contrast, insulin acts as a suppressor. It is well established that carbohydrate and fat metabolism in liver are intimately coupled. The synthesis of fatty acids is maximal in the livers of animals fed a high carbohydrate diet. A reverse situation is rapidly obtained upon administration of glucagon (8). There is general agreement that the limiting step in the synthesis of fatty acids is at the level of acetyl CoA carboxylase. In this study, we have shown that glucagon or epinephrine and insulin exert a reciprocal control over the activity of the key lipogenic enzyme, in addition to the key enzymes of carbohydrate metabolism.

The effects of glucagon and epinephrine on enzyme activity are accompanied by a significant increase in the concentration of cyclic AMP, suggesting that the effects are mediated by this nucleotide, presumably by activation-deactivation of cyclic AMP-dependent protein kinases (40). Indeed, it has been reported that glucagon increases the extent of phosphorylation of the glycolytic enzymes, rendering them less active (41,42). The effect of insulin is less clear. As others have reported (39), insulin does not produce a change in cyclic AMP, suggesting that insulin does not mediate its intracellular action via cyclic AMP. The hormone may exert its effect via several mediators different from c-AMP (43).

It is important to extrapolate the activity of an enzyme assayed in broken-cell preparations to its presumed activity in the intact cell or tissue, particularly when the enzyme is membrane-bound or latent, and hence subject in vivo to a degree of constraint difficult to predict. This problem is further magnified in the liver. Several studies using histochemical techniques or microdissection have found differences in the distribution of enzymes among hepatocytes of the liver acinus (44-47). According to these studies, acinar zone-1 hepatocytes are predominantly engaged in gluconeogenesis, while cells in acinar zone 3 participate predominantly in glycolysis. There is no sharp division between gluconeogenic and glycolytic hepatocytes. Enzymes corresponding to each pathway can be detected in both zones. Only the predominance of one or another rate-limiting enzyme system would make the zone either gluconeogenic or glycolytic. In addition, other parameters that have not been determined at the zone level, such as the concentration of substrates, coenzymes, activators, inhibitors and oxygen in each zone may play important roles in determining these metabolic fluxes in vivo. In our experiments, tissue samples were randomly selected from the periphery of the liver lobes, thus minimizing the variability and the effect of metabolic zonation on the outcome of this study.

CONCLUSIONS

The study demonstrates that insulin acts antagonistically to glucagon and epinephrine in controlling blood glucose concentrations in mammals. The rapid effect of these hormones on blood glucose is, at least in part, explained by alterations in the activities of several strategic glycolytic and gluconeogenic enzyme systems in the liver. Accompanying the rapid changes in enzyme activities are pronounced changes in the flow of glucose carbons via the tricarboxylic acid and the pentose phosphate cycles. The present study suggests that in vivo oxidation of specific carbons of glucose is dependent on the relative concentrations of insulin, glucagon, and epinephrine in the tissues. The study further suggests that the three hormones coordinate complex physiologic mechanisms to prevent or correct hypoglycemia.

RECOMMENDATIONS

The critical biochemical and physiological factors affecting the concentration of plasma glucose in humans subjected to a variety of stresses should be delineated.

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APPENDIX

TABLE 1

Effect of Glucagon, Insulin and Epinephrine on Plasma Glucose Levels[a]

	Hormone			
Time (Min)	None	Glucagon[b]	Insulin[c]	Epinephrine[d]
0	158.4±4.7	160.8±5.7	162.9±4.2	157.9±3.8
5	162.0±5.1	238.2±9.7*	87.3±5.6*	197±11.8*
10	159.0±3.6	272.4±10.3*	63.8±4.6*	201.5±12.6*
20	157.9±4.1	285.9±7.5*	59.4±3.8*	195.7±10.9*
30	160.1±3.8	231.5±8.3*	50.9±7.2*	183.6±7.3*
40	158.7±5.3	238.9±9.8*	57.5±6.9*	189.3±6.2*

[a]Units for mean±SEM values from 6 rats are mg/dl.

[b]dose = 1 mg/kg BW

[c]dose = 0.1 unit/kg BW

[d]dose = 7 ug/kg BW

*Indicates significant difference from zero time, same hormone, $P<0.05$.

TABLE 2

Effect of Glucagon and Insulin on Glucose Oxidation[a]

GROUP	TREATMENT	Minutes after Administration 5	10	20	40	PLASMA GLUCOSE (mg/dl)
			U-^{14}C-Glucose[d]			
1	Control	39.2±6.2	81.8±7.8	187.3±20.1	377.3±46.6	159.9±4.2
2	Glucagon[b]	81.5±10.3*	174.6±19.4*	397.1±40.3*	791.2±50.8*	242.7±5.1*
3	Insulin[c]	67.6±9.4*	149.1±13.7*	280.4±30.3*	635.1±41.5*	56.4±4.6*
4	Glucagon & Insulin	85.1±9.4*	187.5±16.9*	410.6±31.2*	788.9±61.2*	184.8±7.2*
			1-^{14}C-Glucose[e]			
1	Control	69.7±8.9	140.8±15.2	263.1±25.2	516.5±28.1	160.7±5.8
2	Glucagon	60.4±7.1	134.6±18.2	241.7±19.6	529.3±33.1	266.9±10.3*
3	Insulin	150.1±16.8*	270.6±21.8*	510.3±49.7*	981.9±40.8*	48.9±2.7*
4	Glucagon & Insulin	71.3±9.7	149.8±17.6	276.0±20.8	519.1±42.1	188.9±6.5*
			2-^{14}C-Glucose[f]			
1	Control	54.6±8.7	105.0±12.7	227.1±19.6	396.5±40.7	161.3±4.9
2	Glucagon	120.0±10.4*	197.4±13.6	369.1±27.3*	714.8±50.2*	275.4±12.7*
3	Insulin	101.6±9.7*	210.2±18.7*	357.1±20.4*	675.0±54.6*	53.0±3.6*
4	Glucagon & Insulin	112.3±10.3*	201.7±18.5*	389.1±19.4*	681.2±60.4*	191.3±4.8*
			6-^{14}C-Glucose[g]			
1	Control	29.1±6.4	63.8±7.6	101.3±11.9	224.2±30.2	164.7±3.8
2	Glucagon	64.4±8.9*	105.8±11.2*	223.6±18.7*	409.2±39.7*	270.5±6.8*
3	Insulin	28.1±4.2	69.7±8.1	107.3±11.8	248.3±26.1	51.6±7.0*
4	Glucagon & Insulin	58.6±6.2*	127.2±13.4*	210.7±19.4*	395.1±45.2*	185.3±5.6*

[a]Units are DPMs expired X 1000, mean±SEM from 8 rats.
[b]1 mg/kg BW in each experiment.
[c]0.1 unit/kg BW each experiment.
[d]Specific Activity 4.1 mCi/mM, dosage 3.3 uCi/100 g BW.
[e]Specific Activity 3.9 mCi/mM, dosage 2.1 uCi/100 g BW
[f]Specific Activity 3.0 mCi/mM, dosage 2.7 uCi/100 g BW
[g]Specific Activity 3.0 mCi/mM, dosage 3.1 uCi/100 g BW
*Indicates significant difference from control P<0.05, same isotope.

TABLE 3

Effect of Epinephrine[a] on Glucose Oxidation

Group	Treatment	Minutes after administration 5	10	20	40	Plasma Glucose (mg/dl)
			1-^{14}C-Glucose[c]			
1	Control	30.1±4.3[b]	58.2±9.3	112.4±11.6	238.4±11.4	157.2±10.3
2	Epinephrine	28.4±6.1	51.8±6.3	99.2±10.3	229.6±12.8	190.5±8.3*
			2-^{14}C-Glucose[d]			
3	Control	21.6±7.4	54.8±9.3	119.4±15.8	195.3±16.7	160.5±5.8
4	Epinephrine	25.1±5.2	51.6±7.2	124.6±8.7	202.4±14.8	194.1±9.1*
			6-^{14}C-Glucose[e]			
5	Control	10.8±1.3	22.4±4.6	50.3±6.8	119.7±10.4	165.6±7.3
6	Epinephrine	20.4±3.6*	37.8±3.6*	79.1±5.4*	165.1±12.7*	209.4±10.1*

[a] 0.7 ug/100g BW

[b] Values are DPMs expired X 1000, mean ± SEM from 6 rats

[c] Specific activity: 6.9 mCi/mM, Dosage: 1.20 uCi/100g BW

[d] Specific activity: 8.1 mCi/mM, Dosage: 1.62 uCi/100g BW

[e] Specific activity: 9.0 mCi/mM, Dosage: 1.53 uCi/100g BW

* Indicates significant difference from corresponding controls, $P<0.05$

TABLE 4

Effect of Glucagon[a] on 1-^{14}C-Glucose[b] and 6-^{14}C-Glucose[c] Oxidation in Fed and Fasted Rats[d]

Group	Glucose	Nutritional State	Treatment	DPM Expired[e] (Thousands)	Plasma Glucose (mg/dl)
1	1-^{14}C	Fed	Control	350.2±27.8*	160.8±9.3
2	1-^{14}C	Fed	Glucagon	378.4±30.1	264.5±10.8*
3	1-^{14}C	Fasted	Control	150.9±14.6	125.3±4.3
4	1-^{14}C	Fasted	Glucagon	176.4±12.7	130.6±8.2
5	6-^{14}C	Fed	Control	127.2±10.3	165.4±8.2
6	6-^{14}C	Fed	Glucagon	311.3±12.8*	276.9±9.0*
7	6-^{14}C	Fasted	Control	3.9±7	121.8±2.1
8	6-^{14}C	Fasted	Glucagon	38.6±2.3*	135.7±10.3

[a] 1mg/kg BW.

[b] Specific activity: 3.0 Ci/mM, Dosages: 1.71 Ci/100 g BW.

[c] Specific activity: 3.0 Ci/mM, Dosages: 1.62 Ci/100 g BW.

[d] Fasted 48 hours prior to experimentation.

[e] Values are mean±SEM from 6 rats.

* Indicates significant difference from control, $P<0.05$, same isotope, same nutritional state.

TABLE 5

Effect of Glucagon[a] on the Activity of Hepatic Enzymes and Cyclic AMP

	Minutes Following Administration				
Enzymes[b]	0	5	10	20	40
Glucose-6-phosphatase	39±4	59±3*	63±5*	54±3*	41±6
Glucokinase	18±2	7±1*	8±2*	6±1*	10±2*
Fructose-1,6-diphosphatase	40±4	93±3*	67±5*	75±8*	60±5*
Phosphofructokinase	71±7	32±5*	36±4*	31±2*	37±5*
Pyruvate kinase	146±9	101±7*	85±6*	87±5*	73±9*
Phosphoenolpyruvate carboxykinase	58±4	118±7*	127±9*	113±10*	109±10*
Glucose-6-phosphate dehydrogenase	168±13	157±9	152±12	169±7	163±9
Acetyl CoA carboxylase	9±2	3±1*	4±2*	2±1*	3±1*
Cyclic AMP[c]	12±2	49±5*	52±5*	45±6*	34±4*

[a] 1 mg/kg BW
[b] Nanomoles/min/mg protein, mean±SEM from 5 rats.
[c] Picomoles/mg protein, mean±SEM from 5 rats.
*Indicates significant difference from zero time, $P<0.05$.

TABLE 6

Effect of Insulin[a] on the Activity of

Hepatic Enzymes and Cyclic AMP

	Minutes Following Administration				
Enzymes[b]	0	5	10	20	40
Glucose-6-phosphatase	45±6	21±3*	26±2*	28±1*	26±3*
Glucokinase	21±2	42±6*	47±4*	41±4*	39±4*
Fructose-1,6-diphosphatase	47±4	28±3*	25±4*	27±2*	43±6
Phosphofructokinase	65±5	94±6*	93±7*	89±7*	69±6
Pyruvate kinase	153±10	227±13*	230±15*	210±14*	160±15
Phosphoenolpyruvate carboxykinase	63±5	62±4	52±6	59±5	67±4
Glucose-6-phosphate dehydrogenase	159±12	246±16*	263±18*	250±15*	190±18
Acetyl CoA carboxylase	10±2	18±3*	21±4*	19±2*	12±1
Cyclic AMP[c]	14±3	16±2	12±3	15±3	14±2

[a] 0.1 unit/kg

[b] Nanomoles/min/mg protein, mean±SEM from 5 rats.

[c] Picomoles/mg protein, mean±SEM from 5 rats.

*Indicates significant difference from zero time, $P<0.05$.

TABLE 7

Effect of Epinephrine[a] on the Activity of Hepatic Enzymes and Cyclic AMP

	Minutes Following Administration				
Enzymes[b]	0	5	10	20	40
Glucose-6-phosphatase	45±7	52±5	42±6	49±3	54±7
Glucokinase	15±2	6±1*	8±2*	13±4	16±3
Fructose-1,6-diphosphatase	37±5	72±6*	65±4*	42±8	35±7
Phosphofructokinase	62±5	28±3*	25±5*	29±5*	58±7
Pyruvate kinase	163±12	97±10*	89±10*	142±15	153±16
Phosphoenolpyruvate carboxykinase	69±8	105±7*	112±11*	74±5	75±8
Glucose-6-phosphate dehydrogenase	153±14	167±18	156±10	149±16	158±14
Acetyl CoA carboxylase	12±2	5±1*	6±2*	10±4	14±3
Cyclic AMP[c]	9±2	25±3*	18±1*	14±3	8±3

[a] 7 ug/kg BW
[b] Values are nanomoles/min/mg protein, mean±SEM from 5 rats.
[c] Picomoles/mg protein, mean±SEM from 5 rats.
*Indicates significant difference from zero time, $P<0.05$.

FIGURE 1

METABOLISM OF GLUCOSE VIA THE GLYCOLYTIC AND THE PENTOSE PHOSPHATE PATHWAYS

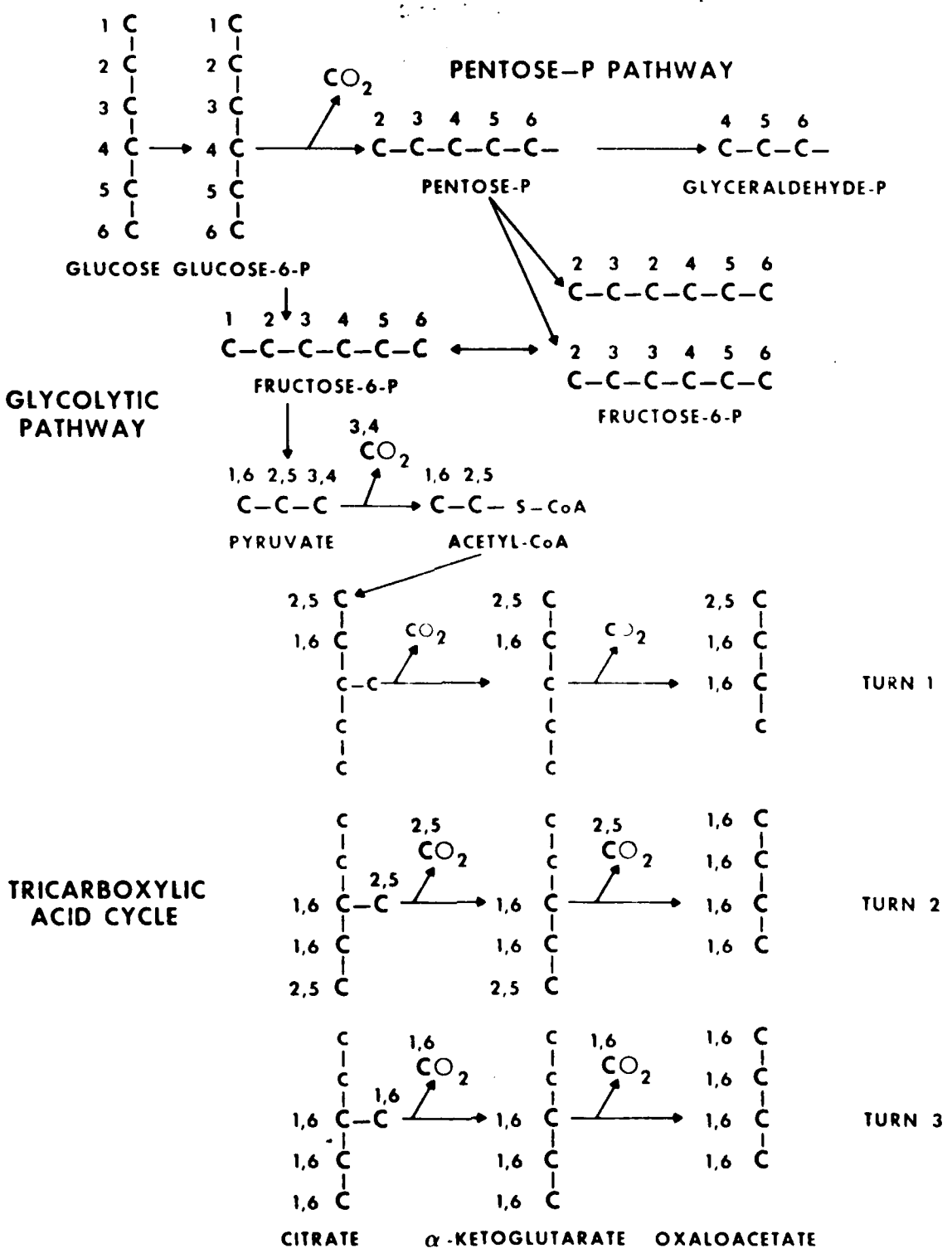


FIGURE 2

INSULIN AND ENZYME ACTIVITY

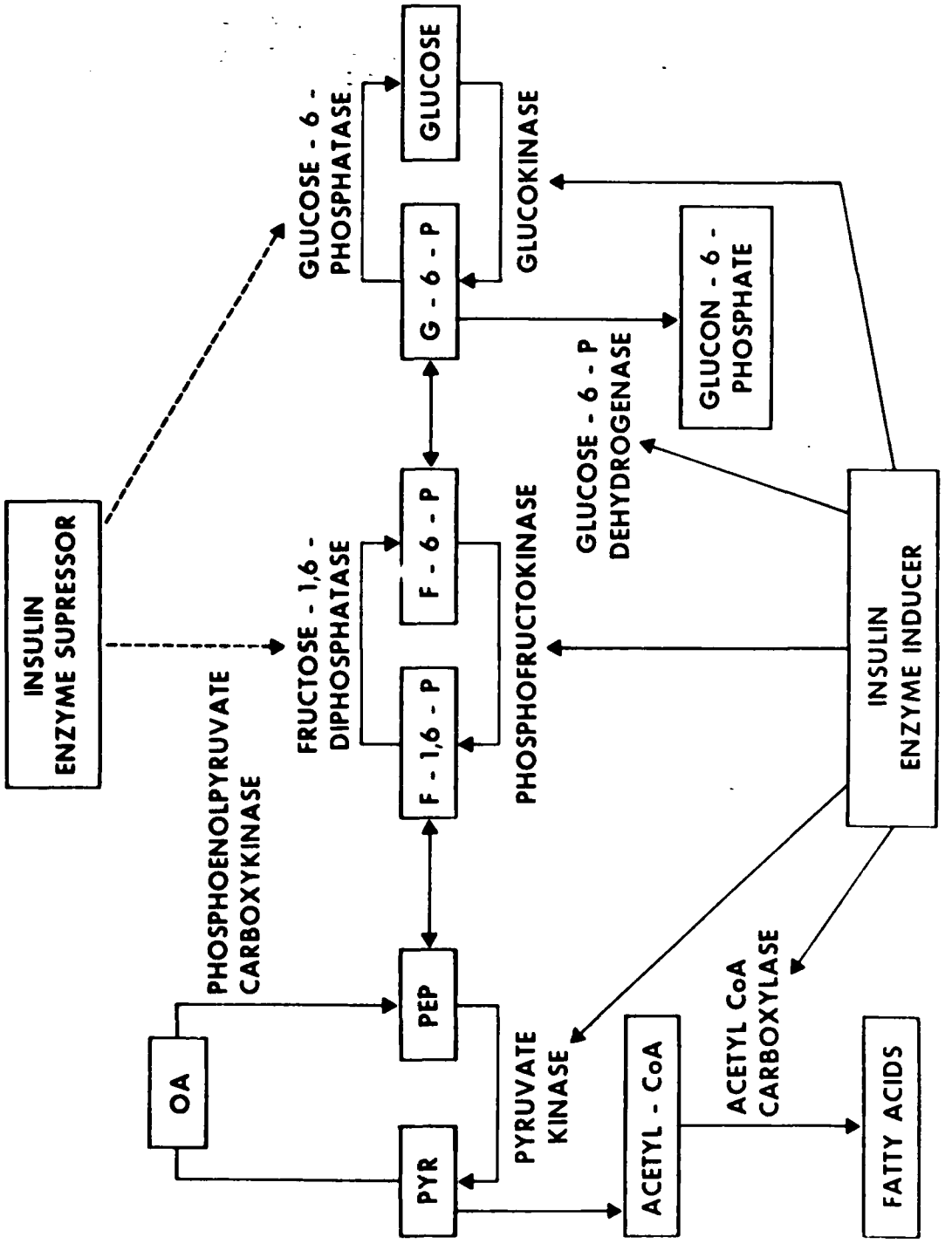


FIGURE 3

GLUCAGON AND ENZYME ACTIVITY

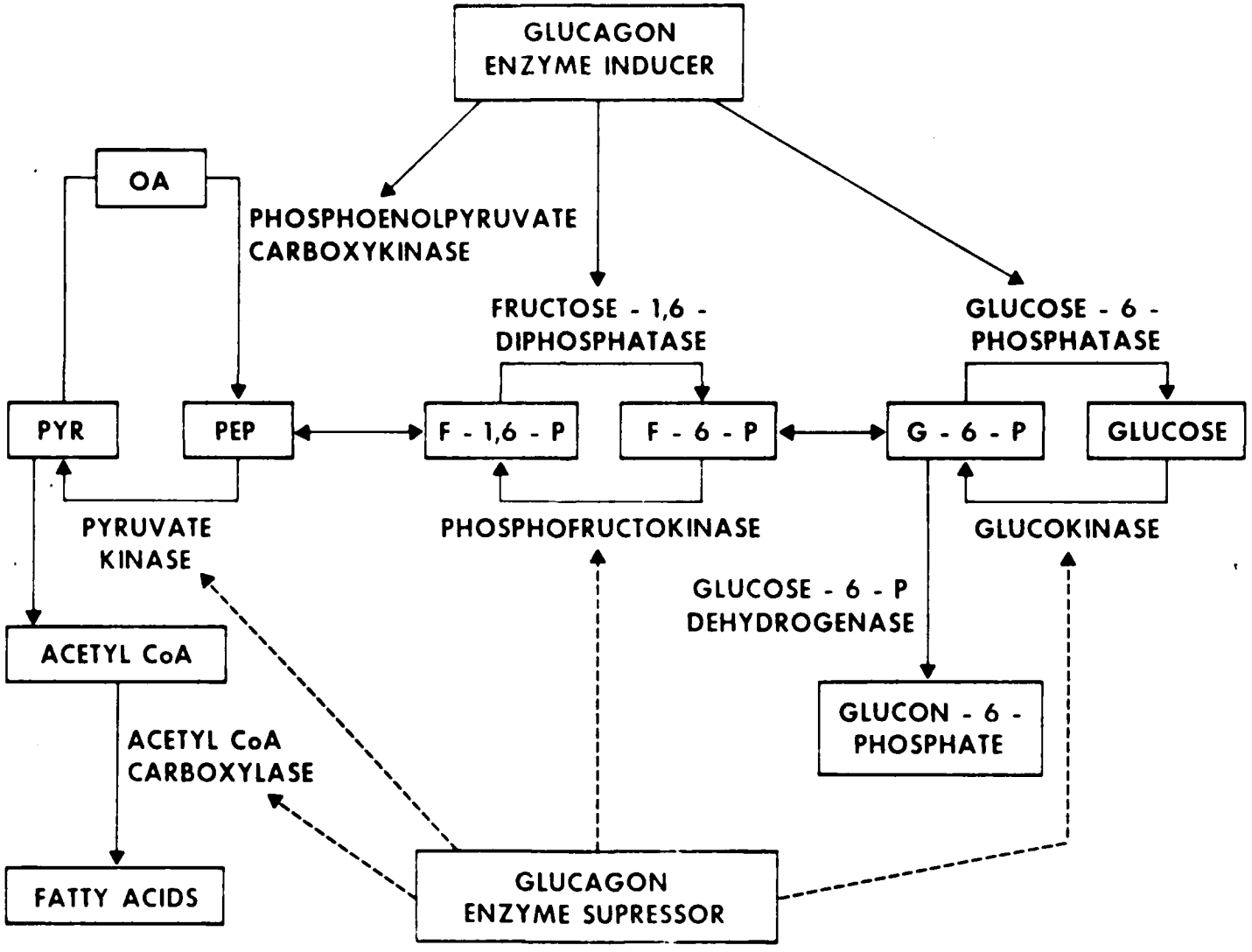
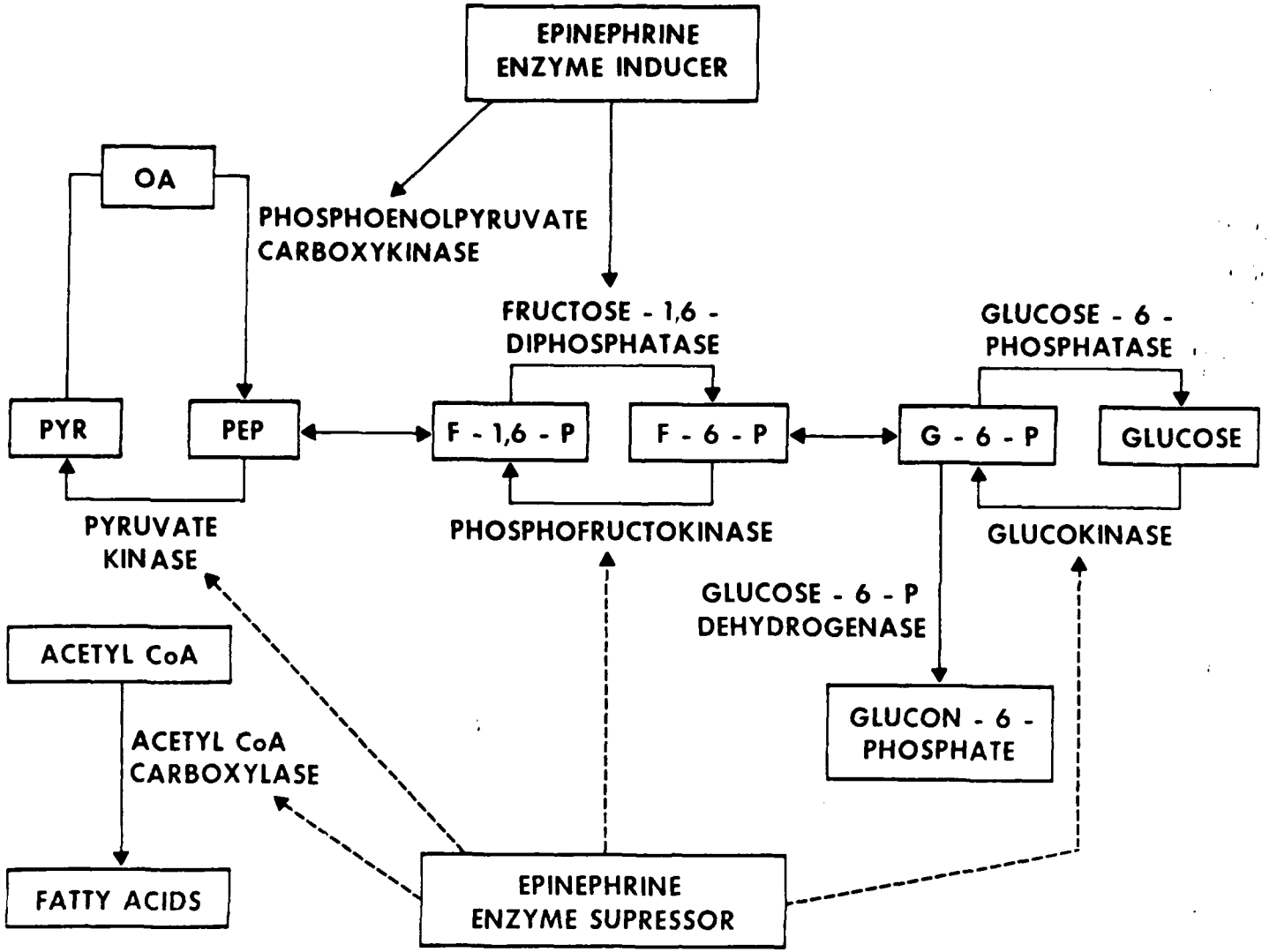


FIGURE 4

EPINEPHRINE AND ENZYME ACTIVITY



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